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(54) Title: REGULATION OF HUMAN SERINE PALMITOYLTRANSFERASE

(57) Abstract: Reagents which regulate human serine palmitoyltransferase and reagents which bind to human serine palmitoyltransferase gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, HIV infection, cancer, obesity, and diabetes.

REGULATION OF HUMAN SERINE PALMITOYLTRANSFERASE

TECHNICAL FIELD OF THE INVENTION

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The invention relates to the area of enzyme regulation. More particularly, the invention relates to the regulation of human serine palmitoyltransferase and its regulation.

BACKGROUND OF THE INVENTION

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Serine palmitoyltransferase (EC 2.3.1.50) catalyzes the formation of a homologue of 3-ketosphinganine from L-serine and a fatty acyl coenzyme A thioester, which is the initial reaction of sphingolipid biosynthesis. Williams *et al.*, *Cancer Res.* 44, 1918-23, 1984. It has recently been proposed that serine palmitoyltransferase is the enzyme which controls *de novo* ceramide synthesis during apoptosis. Perry *et al.*, *J. Biol. Chem.* 275, 9078-84, 2000.

15

Serine palmitoyltransferase has been implicated in cancer. See, e.g., Williams *et al.*, 20 1984; Schroeder *et al.*, *J. Biol. Chem.* 269, 3475-81, 1994. Serine palmitoyltransferase activity also has been implicated in the lipopapoptosis of obese diabetic rats. Shimabukuro *et al.*, *J. Biol. Chem.* 273, 32487-90, 1998.

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Serine palmitoyltransferase including other enzymes of the host cell cholesterol, phospho- as well as sphingolipid biosynthesis have been implicated in various steps within the replicative cycle of Human immunodeficiency virus (HIV). HIV induced cell fusion, nuclear import, DNA integration, chromatin template capacity as well as virion budding from the host cell may be mediated by the lipid environment. Raulin, *J., Progress in Lipid Research* 41, 27-65, 2002.

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There is, therefore, a need in the art to identify related enzymes which can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

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It is an object of the invention to provide reagents and methods of regulating a human serine palmitoyltransferase. This and other objects of the invention are provided by one or more of the embodiments described below.

10 One embodiment of the invention is a serine palmitoyltransferase polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 74% identical to the amino acid sequence shown in SEQ ID NO: 2; and

15

the amino acid sequence shown in SEQ ID NO: 2.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a
20 serine palmitoyltransferase polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 74% identical to the amino acid sequence shown in SEQ ID NO: 2; and

25

the amino acid sequence shown in SEQ ID NO: 2.

Binding between the test compound and the serine palmitoyltransferase polypeptide is detected. A test compound which binds to the serine palmitoyltransferase poly-
30 peptide is thereby identified as a potential agent for decreasing extracellular matrix

degradation. The agent can work by decreasing the activity of the serine palmitoyltransferase.

5 Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a serine palmitoyltransferase polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

10 nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

15 Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the serine palmitoyltransferase through interacting with the serine palmitoyltransferase mRNA.

20 Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a serine palmitoyltransferase polypeptide comprising an amino acid sequence selected from the group consisting of:

25 amino acid sequences which are at least about 74% identical to the amino acid sequence shown in SEQ ID NO: 2; and

the amino acid sequence shown in SEQ ID NO: 2.

30 A serine palmitoyltransferase activity of the polypeptide is detected. A test compound which increases serine palmitoyltransferase activity of the polypeptide relative

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to serine palmitoyltransferase activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases serine palmitoyltransferase activity of the polypeptide relative to serine palmitoyltransferase activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a serine palmitoyltransferase product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

Binding of the test compound to the serine palmitoyltransferase product is detected. A test compound which binds to the serine palmitoyltransferase product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a serine palmitoyltransferase polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1; and

the nucleotide sequence shown in SEQ ID NO: 1.

Serine palmitoyltransferase activity in the cell is thereby decreased.

The invention thus provides a human serine palmitoyltransferase which can be used to identify test compounds which may act, for example, as activators or inhibitors at the enzyme's active site. Human serine palmitoyltransferase and fragments thereof
5 also are useful in raising specific antibodies which can block the enzyme and effectively reduce its activity.

BRIEF DESCRIPTION OF THE DRAWINGS

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Fig. 1 shows the DNA-sequence encoding a serine palmitoyltransferase Polypeptide (SEQ ID NO: 1).

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Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO: 2).

Fig. 3 shows the amino acid sequence of the protein identified by SwissProt Accession No. O15270|LCB2 (SEQ ID NO: 3).

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Fig. 4 shows the amino acid sequence of a serine palmitoyltransferase Polypeptide (SEQ ID NO: 4).

Fig. 5 shows the DNA-sequence encoding a serine palmitoyltransferase Polypeptide (SEQ ID NO: 5).

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Fig. 6 shows the DNA-sequence encoding a serine palmitoyltransferase Polypeptide (SEQ ID NO: 6).

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Fig. 7 shows the DNA-sequence encoding a serine palmitoyltransferase Polypeptide (SEQ ID NO: 7).

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Fig. 8 shows the BLASTP - alignment of genewise_protein_223 (SEQ ID NO: 2) against swiss|O15270|LCB2_HUMAN (SEQ ID NO: 3).

Fig. 9 shows the BLASTP - alignment of genewise_protein_223 (SEQ ID NO: 2) against pdb|1BS0|1BS0-A.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide encoding a serine palmitoyl-transferase polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a serine palmitoyltransferase polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 74% identical to the amino acid sequence shown in SEQ ID NO: 2; and the amino acid sequence shown in SEQ ID NO: 2.

b) a polynucleotide comprising the sequence of SEQ ID NO: 1.

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

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Furthermore, it has been discovered by the present applicant that a novel serine palmitoyltransferase, particularly a human serine palmitoyltransferase, can be used in therapeutic methods to treat HIV infection, cancer, obesity or diabetes.

5 Human serine palmitoyltransferase comprises the amino acid sequence shown in SEQ ID NO: 2. Human serine palmitoyltransferase is 73% identical over 230 amino acids to the human protein identified with SwissProt Accession No. O15270 (SEQ ID NO: 3) and annotated as "SERINE PALMITOYLTRANSFERASE 2 (EC 2.3.1.50)" (Fig. 8). Human serine palmitoyltransferase also is 32% identical over
10 116 amino acids to pdb|1BS0|1BS0-A (Fig. 9).

A coding sequence for human serine palmitoyltransferase is shown in SEQ ID NO: 1. This sequence is located on chromosome 20. Related EST sequences (SEQ ID NOS: 4-8) are expressed in HeLaS3 cells, tumor (mouse), pancreatic islets, brain, and
15 germinal center B cells.

Human serine palmitoyltransferase is expected to be useful for the same purposes as previously identified serine palmitoyltransferases. Thus, human serine palmitoyltransferase can be used in therapeutic methods to treat disorders such as HIV
20 infection, cancer, obesity, and diabetes. Human serine palmitoyltransferase also can be used to screen for human serine palmitoyltransferase activators and inhibitors.

Polypeptides

25 Human serine palmitoyltransferase polypeptides according to the invention comprise at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, or 230 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO: 2 or a biologically active variant thereof, as defined below. A human serine palmitoyltransferase polypeptide of the invention therefore can be a portion of a human serine
30 palmitoyltransferase, a full-length human serine palmitoyltransferase, or a fusion protein comprising all or a portion of a human serine palmitoyltransferase.

Biologically Active Variants

Human serine palmitoyltransferase polypeptide variants which are biologically
5 active, *e.g.*, retain an aminotransferase activity, also are human serine palmitoyl-
transferase polypeptides. Preferably, naturally or non-naturally occurring serine
palmitoyltransferase polypeptide variants have amino acid sequences which are at
least about 74, 75, 80, 85, 90, 96, 96, or 98% identical to the amino acid sequence
shown in SEQ ID NO: 2 or a fragment thereof. Percent identity between a putative
10 polypeptide variant and an amino acid sequence of SEQ ID NO: 2 is determined by
conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48:603
(1986), and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1992).
Briefly, two amino acid sequences are aligned to optimize the alignment scores using
a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62"
15 scoring matrix of Henikoff and Henikoff (*ibid.*). Those skilled in the art appreciate
that there are many established algorithms available to align two amino acid
sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a
suitable protein alignment method for examining the level of identity shared by an
amino acid sequence disclosed herein and the amino acid sequence of a putative
20 variant. The FASTA algorithm is described by Pearson and Lipman, Proc. Nat'l Acad.
Sci. USA 85:2444(1988), and by Pearson, Meth. Enzymol. 183:63 (1990). Briefly,
FASTA first characterizes sequence similarity by identifying regions shared by the
query sequence (*e.g.* SEQ ID NO: 2) and a test sequence that have either the highest
density of identities (if the *ktup* variable is 1) or pairs of identities (if *ktup*=2),
25 without considering conservative amino acid substitutions, insertions, or deletions.
The ten regions with the highest density of identities are then rescored by comparing
the similarity of all paired amino acids using an amino acid substitution matrix, and
the ends of the regions are "trimmed" to include only those residues that contribute to
the highest score. If there are several regions with scores greater than the "cutoff"
30 value (calculated by a predetermined formula based upon the length of the sequence
and the *ktup* value), then the trimmed initial regions are examined to determine

whether the regions can be joined to form approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, J. Mol. Biol. 48:444 (1970); Sellers, SIAM J. Appl. Math. 26:787 (1974)), which allows for amino acid insertions and deletions. Preferred parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, Meth. Enzymol. 183:63 (1990). FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as default.

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a serine palmitoyltransferase polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active polypeptide can readily be determined by assaying for aminotransferase activity, as described, for example, in Kontani *et al.*, *Biochim. Biophys. Acta* 1156, 161-66, 1993.

Fusion Proteins

Fusion proteins are useful for generating antibodies against serine palmitoyltransferase amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of a serine palmitoyltransferase polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

10

A serine palmitoyltransferase fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, or 230 contiguous amino acids of SEQ ID NO: 2 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length serine palmitoyltransferase.

15

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β -galactosidase, β -glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the serine palmitoyltransferase polypeptide-encoding sequence and the heterologous protein sequence, so that the desired polypeptide can be cleaved and purified away from the heterologous moiety.

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A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from the complement of SEQ ID NO: 1 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human serine palmitoyltransferase polypeptide can be obtained using serine palmitoyltransferase polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of serine palmitoyltransferase polypeptide, and expressing the cDNAs as is known in the art.

Polynucleotides

A serine palmitoyltransferase polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a serine palmitoyltransferase polypeptide. A coding sequence for serine palmitoyltransferase shown in SEQ ID NO: 2 is shown in SEQ ID NO: 1.

Degenerate nucleotide sequences encoding human serine palmitoyltransferase polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NO: 1 or its complement also are serine palmitoyltransferase polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of serine palmitoyltransferase polynucleotides which encode biologically active serine palmitoyltransferase polypeptides also are serine palmitoyltransferase polynucleotides. Polynucleotides comprising at least 6, 7, 8, 9, 10, 12, 15, 18, 20, or 25 contiguous nucleotides of SEQ ID NO: 1 or its complement also are serine palmitoyltransferase polynucleotides. Such polynucleotides can be used, for example, as hybridization probes or antisense oligonucleotides.

15

Identification of Polynucleotide Variants and Homologs

Variants and homologs of the polynucleotides described above also are serine palmitoyltransferase polynucleotides. Typically, homologous polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known Serine palmitoyltransferase polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the serine palmitoyltransferase polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA

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expression libraries from other species, such as mice, monkeys, or yeast. Human variants of Serine palmitoyltransferase polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol.* 81, 123 (1973)). Variants of human serine palmitoyltransferase polynucleotides or serine palmitoyltransferase polynucleotides of other species can therefore be identified by hybridizing a putative homologous polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO: 1 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to serine palmitoyltransferase polynucleotides or their complements following stringent hybridization and/or wash conditions also are serine palmitoyltransferase polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a polynucleotide having a nucleotide sequence shown in SEQ ID NO: 1 or the complement thereof and a polynucleotide sequence which is at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* 48, 1390 (1962):

$$T_m = 81.5^\circ\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l,$$

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65°C, or 50% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2X SSC at 65°C.

5 Preparation of Polynucleotides

A serine palmitoyltransferase polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated serine palmitoyltransferase polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises aminotransferase-like nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

Human serine palmitoyltransferase cDNA molecules can be made with standard molecular biology techniques, using human serine palmitoyltransferase mRNA as a template. Human serine palmitoyltransferase cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize serine palmitoyltransferase polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a polypeptide having, for example, an amino acid sequence shown in SEQ ID NO: 2 or a biologically active variant thereof.

Extending Polynucleotides

5 Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic.* 2, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences
10 are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

15 Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res.* 16, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the
20 target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR ampli-
25 fication of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic.* 1, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

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Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res.* 19, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

25

Obtaining Polypeptides

Human serine palmitoyltransferase polypeptides can be obtained, for example, by purification from human cells, by expression of serine palmitoyltransferase polynucleotides, or by direct chemical synthesis.

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Protein Purification

Human serine palmitoyltransferase polypeptides can be purified from any cell which expresses the enzyme, including host cells which have been transfected with serine
5 palmitoyltransferase expression constructs. Infant brain and kidney provide especially useful sources of serine palmitoyltransferase polypeptides. A purified serine palmitoyltransferase polypeptide is separated from other compounds which normally associate with the serine palmitoyltransferase polypeptide in the cell, such as certain
10 proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified serine palmitoyltransferase polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99%
15 pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of Polynucleotides

To express a human serine palmitoyltransferase polynucleotide, the polynucleotide
20 can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding serine palmitoyltransferase polypeptides and appropriate transcriptional and translational control elements. These methods
25 include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

30 A variety of expression vector/host systems can be utilized to contain and express sequences encoding a serine palmitoyltransferase polypeptide. These include, but are

not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (e.g., baculovirus), plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial
5 expression vectors (e.g., Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with
10 host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the
15 BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) can be cloned into the vector. In mammalian
20 cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a serine palmitoyltransferase polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

25 Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the serine palmitoyltransferase polypeptide. For example, when a large quantity of a polypeptide is needed for the induction of antibodies, vectors
30 which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and

expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem.* 264, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol.* 153, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding serine palmitoyltransferase polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J.* 6, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J.* 3, 1671-1680, 1984; Broglie *et al.*, *Science* 224, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ.* 17, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (e.g.,

Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

5 An insect system also can be used to express a serine palmitoyltransferase polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding serine palmitoyltransferase polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter.
10 Successful insertion of serine palmitoyltransferase polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which serine palmitoyltransferase polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci.* 91, 3224-3227, 1994).

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Mammalian Expression Systems

A number of viral-based expression systems can be used to express serine palmitoyltransferase polypeptides in mammalian host cells. For example, if an adenovirus is
20 used as an expression vector, sequences encoding serine palmitoyltransferase polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a serine palmitoyltransferase polypeptide in infected host cells
25 (Logan & Shenk, *Proc. Natl. Acad. Sci.* 81, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments
30 of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are

constructed and delivered to cells via conventional delivery methods (e.g., liposomes, polycationic amino polymers, or vesicles).

5 Specific initiation signals also can be used to achieve more efficient translation of sequences encoding serine palmitoyltransferase polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a serine palmitoyltransferase polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases
10 where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be
15 enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ.* 20, 125-162, 1994).

Host Cells

20 A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed serine palmitoyltransferase polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation,
25 and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801
30 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express serine palmitoyltransferase polypeptides can be transformed using expression vectors which can contain viral
5 origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which
10 successfully express the introduced serine palmitoyltransferase sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

15 Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell* 11, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell* 22, 817-23, 1980) genes which can be employed in *tk* or *aprt* cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as
20 the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci.* 77, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol.* 150, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes
25 have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci.* 85, 8047-51, 1988). Visible markers such as anthocyanins, β -glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount
30 of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol.* 55, 121-131, 1995).

Detecting Expression

Although the presence of marker gene expression suggests that the serine palmitoyl-
transferase polynucleotide is also present, its presence and expression may need to be
5 confirmed. For example, if a sequence encoding a serine palmitoyltransferase
polypeptide is inserted within a marker gene sequence, transformed cells containing
sequences which encode a serine palmitoyltransferase polypeptide can be identified
by the absence of marker gene function. Alternatively, a marker gene can be placed
10 in tandem with a sequence encoding a serine palmitoyltransferase polypeptide under
the control of a single promoter. Expression of the marker gene in response to
induction or selection usually indicates expression of the serine palmitoyltransferase
polynucleotide.

15 Alternatively, host cells which contain a serine palmitoyltransferase polynucleotide
and which express a serine palmitoyltransferase polypeptide can be identified by a
variety of procedures known to those of skill in the art. These procedures include,
but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay
or immunoassay techniques which include membrane, solution, or chip-based
20 technologies for the detection and/or quantification of nucleic acid or protein. For
example, the presence of a polynucleotide sequence encoding a serine palmitoyl-
transferase polypeptide can be detected by DNA-DNA or DNA-RNA hybridization
or amplification using probes or fragments or fragments of polynucleotides encoding
a serine palmitoyltransferase polypeptide. Nucleic acid amplification-based assays
25 involve the use of oligonucleotides selected from sequences encoding a serine
palmitoyltransferase polypeptide to detect transformants which contain a serine
palmitoyltransferase polynucleotide.

A variety of protocols for detecting and measuring the expression of a serine
30 palmitoyltransferase polypeptide, using either polyclonal or monoclonal antibodies
specific for the polypeptide, are known in the art. Examples include enzyme-linked

immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a serine palmitoyltransferase polypeptide can be used, or a competitive binding assay can be employed. These and
5 other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med.* 158, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the
10 art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding serine palmitoyltransferase polypeptides include oligo-labeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a serine palmitoyltransferase poly-
15 peptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham, Pharmacia Biotech, Promega,
20 and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

25 Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a serine palmitoyl-transferase polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a
30 transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art,

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expression vectors containing polynucleotides which encode serine palmitoyltransferase polypeptides can be designed to contain signal sequences which direct secretion of soluble serine palmitoyltransferase polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound serine palmitoyltransferase polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a serine palmitoyltransferase polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the serine palmitoyltransferase polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a serine palmitoyltransferase polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif.* 3, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the serine palmitoyltransferase polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol.* 12, 441-453, 1993.

Chemical Synthesis

Sequences encoding a serine palmitoyltransferase polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al.* *Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a serine palmitoyltransferase polypeptide itself can be

produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc.* 85, 2149-2154, 1963; Roberge *et al.*, *Science* 269, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis
5 can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of serine palmitoyltransferase polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

10 The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, *PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES*, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic serine palmitoyltransferase polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see*
15 Creighton, *supra*). Additionally, any portion of the amino acid sequence of the serine palmitoyltransferase polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

20 Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce serine palmitoyltransferase polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular
25 prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

30 The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter serine palmitoyltransferase polypeptide-encoding

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sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

10

Any type of antibody known in the art can be generated to bind specifically to an epitope of a serine palmitoyltransferase polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, F(ab')₂, and Fv, which are capable of binding an epitope of a serine palmitoyltransferase polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, e.g., at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a serine palmitoyltransferase polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to a serine palmitoyltransferase polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay.

Preferably, antibodies which specifically bind to serine palmitoyltransferase polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a serine palmitoyltransferase polypeptide from solution.

5 Human serine palmitoyltransferase polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a serine palmitoyltransferase polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to
10 increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (e.g., aluminum hydroxide), and surface active substances (e.g. lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

15 Monoclonal antibodies which specifically bind to a serine palmitoyltransferase polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma
20 technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature* 256, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods* 81, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci.* 80, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol.* 62, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the
25 splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci.* 81, 6851-6855, 1984; Neuberger *et al.*, *Nature* 312, 604-608, 1984; Takeda *et al.*, *Nature* 314, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response
30 against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or

may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a serine palmitoyltransferase polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to serine palmitoyltransferase polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton, *Proc. Natl. Acad. Sci.* 88, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev.* 5, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol.* 15, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem.* 269, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer* 61, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth.* 165, 81-91).

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Antibodies which specifically bind to serine palmitoyltransferase polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci.* 86, 3833-3837, 1989; Winter *et al.*, *Nature* 349, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a serine palmitoyltransferase polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of serine palmitoyltransferase gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated

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synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamdate, carboxymethyl esters, carbonates, and phosphate triesters. See Brown, *Meth. Mol. Biol.* 20, 1-8, 1994; Sonveaux, *Meth. Mol. Biol.* 26, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev.* 90, 543-583, 1990.

Modifications of serine palmitoyltransferase gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the serine palmitoyltransferase gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, *MOLECULAR AND IMMUNOLOGIC APPROACHES*, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a serine palmitoyltransferase polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a serine palmitoyltransferase polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent serine palmitoyltransferase nucleotides, can provide sufficient targeting specificity for serine palmitoyltransferase mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in

length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular serine palmitoyl-transferase polynucleotide sequence.

5

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a serine palmitoyltransferase polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine
10 moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well
15 known in the art. See, e.g., Agrawal *et al.*, *Trends Biotechnol.* 10, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev.* 90, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett.* 215, 3539-3542, 1987.

Ribozymes

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Ribozymes are RNA molecules with catalytic activity. See, e.g., Cech, *Science* 236, 1532-1539; 1987; Cech, *Ann. Rev. Biochem.* 59, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol.* 2, 605-609; 1992, Couture & Stinchcomb, *Trends Genet.* 12, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence,
25 as is known in the art (e.g., Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide
30 sequences.

The coding sequence of a serine palmitoyltransferase polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the serine palmitoyltransferase polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (see Haseloff *et al.* *Nature* 334, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 10 321,201).

Specific ribozyme cleavage sites within a serine palmitoyltransferase RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate serine palmitoyltransferase RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease serine palmitoyltransferase expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate

element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

5

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

10

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human serine palmitoyltransferase. Such genes may represent genes that are differentially expressed in disorders including, but not limited to, HIV infection, cancer, obesity, and diabetes. Further, such genes may represent genes that are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human serine palmitoyltransferase gene or gene product may itself be tested for differential expression.

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The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

30

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique that does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples that represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 85, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature* 308, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 2825, 1984), and, preferably, differential display (Liang & Pardee, *Science* 257, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human serine palmitoyltransferase. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human serine palmitoyltransferase. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human serine palmitoyltransferase gene or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides assays for screening test compounds which bind to or modulate the activity of a serine palmitoyltransferase polypeptide or a serine palmitoyltransferase polynucleotide. A test compound preferably binds to a serine palmitoyltransferase polypeptide or polynucleotide. More preferably, a test compound decreases or increases serine palmitoyltransferase by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. See Lam, *Anticancer Drug Des.* 12, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (see, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90, 6909, 1993; Erb *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 91, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem.* 37, 2678, 1994; Cho *et al.*, *Science* 261, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2061; Gallop *et al.*, *J.*

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Med. Chem. 37, 1233, 1994). Libraries of compounds can be presented in solution (see, e.g., Houghten, *BioTechniques* 13, 412-421, 1992), or on beads (Lam, *Nature* 354, 82-84, 1991), chips (Fodor, *Nature* 364, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89, 1865-1869, 1992), or phage (Scott & Smith, *Science* 249, 386-390, 1990; Devlin, *Science* 249, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci.* 97, 6378-6382, 1990; Felici, *J. Mol. Biol.* 222, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to serine palmitoyltransferase polypeptides or polynucleotides or to affect serine palmitoyltransferase activity or serine palmitoyltransferase gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 μ l. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 19, 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

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Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity* 2, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies, for example, the active site of the serine palmitoyltransferase polypeptide, such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules.

In binding assays, either the test compound or the serine palmitoyltransferase polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemi-

luminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the serine palmitoyltransferase polypeptide can then be accomplished, for example, by direct counting of radioemission, by scintillation counting, or by determining conversion
5 of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a serine palmitoyltransferase polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a serine
10 palmitoyltransferase polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a serine palmitoyltransferase polypeptide (McConnell *et al.*, *Science* 257,
15 1906-1912, 1992).

Determining the ability of a test compound to bind to a serine palmitoyltransferase polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem.* 63,
20 2338-2345, 1991; and Szabo *et al.*, *Curr. Opin. Struct. Biol.* 5, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

25

In yet another aspect of the invention, a serine palmitoyltransferase polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell* 72, 223-232, 1993; Madura *et al.*, *J. Biol. Chem.* 268, 12046-12054, 1993; Bartel *et al.*, *BioTechniques* 14, 920-924, 1993; Iwabuchi
30 *et al.*, *Oncogene* 8, 1693-1696, 1993; and Brent W094/10300), to identify other

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proteins which bind to or interact with the serine palmitoyltransferase polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a serine palmitoyltransferase polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the serine palmitoyltransferase polypeptide.

It may be desirable to immobilize either the serine palmitoyltransferase polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the serine palmitoyltransferase polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the enzyme polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are

preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a v enzyme polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and
5 microcentrifuge tubes.

In one embodiment, the serine palmitoyltransferase polypeptide is a fusion protein comprising a domain that allows the serine palmitoyltransferase polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can
10 be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed serine palmitoyltransferase polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation,
15 the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

20 Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a serine palmitoyltransferase polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated serine palmitoyltransferase polypeptides (or polynucleotides) or test compounds can be
25 prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a serine palmitoyltransferase polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding
30 site, such as the active site of the polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the serine palmitoyltransferase polypeptide or test
5 compound, enzyme-linked assays which rely on detecting an activity of the serine palmitoyltransferase polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a serine palmitoyltransferase polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which
10 comprises a serine palmitoyltransferase polypeptide or polynucleotide can be used in a cell-based assay system. A serine palmitoyltransferase polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a serine palmitoyltransferase
15 polypeptide or polynucleotide is determined as described above.

Enzyme Assays

Test compounds can be tested for the ability to increase or decrease the serine
20 palmitoyltransferase activity of a human serine palmitoyltransferase-like enzyme polypeptide. Enzyme activity can be measured, for example, as described in Williams *et al.*, *Arch. Biochem. Biophys.* 228, 282-91, 1984; Williams *et al.*, *Cancer Res.* 44, 1918-23, 1984; Weiss & Stoffel, *Eur. J. Biochem.* 249, 239-47, 1997; Zweerink *et al.*, *J. Biol. Chem.* 267, 25032-38, 1992; Mandon *et al.*, *Eur. J. Biochem.*
25 198, 667-74, 1991; Holleran *et al.*, *J. Lipid Res.* 31, 1655-61, 1990; Hanada *et al.*, *FEBS Lett.* 474, 63-65, 2000; Sandvig *et al.*, *Mol. Biol. Cell* 7, 1391-404, 1996; Hanada *et al.*, *Biochem. Pharmacol.* 59, 1211-16, 2000. See also Dickson *et al.*, *Methods Enzymol.* 311, 3-9, 2000.

30 Enzyme assays can be carried out after contacting either a purified serine palmitoyltransferase-like enzyme polypeptide, a cell membrane preparation, or an

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intact cell with a test compound. A test compound which decreases enzymatic activity of a serine palmitoyltransferase-like enzyme polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for decreasing serine palmitoyltransferase-like enzyme activity. A test compound which increases enzymatic activity of a human serine palmitoyltransferase-like enzyme polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential therapeutic agent for increasing human serine palmitoyltransferase-like enzyme activity.

10 Gene Expression

In another embodiment, test compounds which increase or decrease serine palmitoyltransferase gene expression are identified. A serine palmitoyltransferase polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the v enzyme polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

25

The level of v enzyme mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a serine palmitoyltransferase polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry.

30

Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a serine palmitoyltransferase polypeptide.

5 Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a serine palmitoyltransferase polynucleotide can be used in a cell-based assay system. The serine palmitoyltransferase polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as
10 CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered
15 to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a serine palmitoyltransferase polypeptide, serine palmitoyltransferase polynucleotide, ribozymes or antisense oligonucleotides, antibodies which specifically bind to a serine palmitoyltransferase polypeptide, or mimetics, activators, or inhibitors of a serine palmitoyltransferase polypeptide
20 activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

25 In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be
30 administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal,

subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be

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dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated
5 in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection
10 suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated
15 solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a
20 manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in
25 aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

30 Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co.,

Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

5

Therapeutic Indications and Methods

Human serine palmitoyltransferase can be regulated to treat HIV infection, cancer, obesity, and diabetes.

10

Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug-resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

20

Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to these agents. Thus, the therapeutic indices for traditional anti-cancer therapies rarely exceed 2.0.

25

The advent of genomics-driven molecular target identification has opened up the possibility of identifying new cancer-specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor-associated genes and their products can be tested for their role(s) in

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disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

5 Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high-throughput molecular screening programs to identify chemical modulators of their biochemical activities. Activators and/or anta

10 protein activity can be identified in this manner and subsequently tested in cellular and *in vivo* disease models for anti-cancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and toxicological analyses form the basis for drug development and subsequent testing in humans.

15 Human serine palmitoyltransferase can be regulated to treat cancer. For example, alanine-glyoxylate aminotransferase activity is inactivated by the chemotherapeutic agents 5-fluorouracil and 6-azauracil, which are chemotherapeutic reagents used to cancer. Kontani *et al.*, 1993. Thus, inactivation of serine palmitoyltransferase can be

20 inactivated or its expression decreased to treat cancer.

Obesity and overweight are defined as an excess of body fat relative to lean body mass. An increase in caloric intake or a decrease in energy expenditure or both can bring about this imbalance leading to surplus energy being stored as fat. Obesity is

25 associated with important medical morbidities and an increase in mortality. The causes of obesity are poorly understood and may be due to genetic factors, environmental factors or a combination of the two to cause a positive energy balance. In contrast, anorexia and cachexia are characterized by an imbalance in energy intake versus energy expenditure leading to a negative energy balance and weight loss.

30 Agents that either increase energy expenditure and/or decrease energy intake, absorption or storage would be useful for treating obesity, overweight, and associated

comorbidities. Agents that either increase energy intake and/or decrease energy expenditure or increase the amount of lean tissue would be useful for treating cachexia, anorexia and wasting disorders.

- 5 This gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity, overweight, anorexia, cachexia, wasting disorders, appetite suppression, appetite enhancement, increases or decreases in satiety, modulation of body weight, and/or other eating disorders such as bulimia. Also this gene, translated proteins and agents which modulate this gene or portions of
- 10 the gene or its products are useful for treating obesity/overweight-associated comorbidities including hypertension, type 2 diabetes, coronary artery disease, hyperlipidemia, stroke, gallbladder disease, gout, osteoarthritis, sleep apnea and respiratory problems, some types of cancer including endometrial, breast, prostate, and colon cancer, thrombotic disease, polycystic ovarian syndrome, reduced fertility,
- 15 complications of pregnancy, menstrual irregularities, hirsutism, stress incontinence, and depression.

- Diabetes mellitus is a common metabolic disorder characterized by an abnormal elevation in blood glucose, alterations in lipids and abnormalities (complications) in
- 20 the cardiovascular system, eye, kidney and nervous system. Diabetes is divided into two separate diseases: type 1 diabetes (juvenile onset); which results from a loss of cells which make and secrete insulin, and type 2 diabetes (adult onset), which is caused by a defect in insulin secretion and a defect in insulin action.

- 25 Type 1 diabetes is initiated by an autoimmune reaction that attacks the insulin secreting cells (beta cells) in the pancreatic islets. Agents that prevent this reaction from occurring or that stop the reaction before destruction of the beta cells has been accomplished are potential therapies for this disease. Other agents that induce beta cell proliferation and regeneration also are potential therapies.

Type II diabetes is the most common of the two diabetic conditions (6% of the population). The defect in insulin secretion is an important cause of the diabetic condition and results from an inability of the beta cell to properly detect and respond to rises in blood glucose levels with insulin release. Therapies that increase the response by the beta cell to glucose would offer an important new treatment for this disease.

The defect in insulin action in Type II diabetic subjects is another target for therapeutic intervention. Agents that increase the activity of the insulin receptor in muscle, liver, and fat will cause a decrease in blood glucose and a normalization of plasma lipids. The receptor activity can be increased by agents that directly stimulate the receptor or that increase the intracellular signals from the receptor. Other therapies can directly activate the cellular end process, *i.e.* glucose transport or various enzyme systems, to generate an insulin-like effect and therefore a produce beneficial outcome. Because overweight subjects have a greater susceptibility to Type II diabetes, any agent that reduces body weight is a possible therapy.

Both Type I and Type diabetes can be treated with agents that mimic insulin action or that treat diabetic complications by reducing blood glucose levels. Likewise, agents that reduces new blood vessel growth can be used to treat the eye complications that develop in both diseases.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an anti-sense nucleic acid molecule, a specific antibody, ribozyme, or a serine palmitoyl-transferase polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention

pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

5 A reagent which affects serine palmitoyltransferase activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce serine palmitoyltransferase activity. The reagent preferably binds to an expression product of a human serine palmitoyltransferase gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can
10 then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about
15 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain,
20 lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about
25 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably
30 between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol.

5 Optionally, a liposome comprises a compound capable of targeting the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or

10 ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μ g to about 10 μ g of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μ g to about 5 μ g of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μ g of polynucleotides is combined

15 with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al.* *Trends in Biotechnol.* 11, 202-05 (1993);

20 Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem.* 263, 621-24 (1988); Wu *et al.*, *J. Biol. Chem.* 269, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem.* 266, 338-42 (1991).

25 Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases enzyme activity relative to the serine

30 palmitoyltransferase activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50} .

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 µg to about 50 µg/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a serine palmitoyltransferase gene or the activity of a serine palmitoyltransferase polypeptide by at least about 10, preferably

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about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a serine palmitoyltransferase gene or the activity of a serine palmitoyltransferase polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to serine palmitoyltransferase-specific mRNA, quantitative RT-PCR, immunologic detection of a serine palmitoyltransferase polypeptide, or measurement of serine palmitoyltransferase activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

Human serine palmitoyltransferase also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences which encode the enzyme. For example, differences can be determined between the cDNA or genomic sequence encoding serine palmitoyltransferase in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

10

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g., Myers et al., Science 230, 1242, 1985*). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g., Cotton et al., Proc. Natl. Acad. Sci. USA 85, 4397-4401, 1985*). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

25

Altered levels of a serine palmitoyltransferase also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

30

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following
5 specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

10 *Detection of serine palmitoyltransferase-like enzyme activity*

The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-serine palmitoyltransferase-like enzyme polypeptide obtained is transfected into human embryonic kidney 293 cells. From these
15 cells extracts are obtained and SPT activity is assayed in a 300- μ l volume containing 50 mM HEPES, 8.3, 50 mM pyridoxal phosphate, 2 mM serine (20 μ Ci/ml) 0.2 mM palmitoyl CoA, 1 mM NADPH, 2.4 mM glucose-6-phosphate, and 10 units of glucose-6-phosphate dehydrogenase. The reaction is initiated by adding 0.5-1 mg of the cell extract, and after 10 min at 37°C, it is terminated by the addition of 100 μ l of
20 2 M NH₄OH and 0.75 ml of CHCl₃:MeOH (1:2). After vortexing, an additional 0.75 ml of CHCl₃:MeOH (1:2), 1 ml of CHCl₃, and 2 ml of 0.5 M NH₄OH are added with vortexing after each addition. After brief centrifugation to separate the phases, the top layer is aspirated off. The organic phase is washed three times (or until clear) with 30 mM KCl, and a fixed volume is dried under N₂ and resuspended
25 in scintillation fluid for counting. SPT activity is expressed in pmol of serine converted per mg of the cell extract per min. It is shown that the polypeptide of SEQ ID NO: 2 has a serine palmitoyltransferase-like enzyme activity.

EXAMPLE 2

Expression of recombinant human serine palmitoyltransferase

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The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human serine palmitoyltransferase polypeptides in yeast. The serine palmitoyltransferase-encoding DNA sequence is derived from SEQ ID NO: 1. Before insertion into vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple cloning site of pPICZ B with the corresponding restriction enzymes the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human serine palmitoyltransferase polypeptide is obtained.

EXAMPLE 3

Identification of test compounds that bind to serine palmitoyltransferase polypeptides

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Purified serine palmitoyltransferase polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well

microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Human serine palmitoyltransferase polypeptides comprise the amino acid sequence shown in SEQ ID NO: 2. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a serine palmitoyltransferase polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to a serine palmitoyltransferase polypeptide.

EXAMPLE 4

15

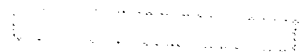
Identification of a test compound which decreases serine palmitoyltransferase gene expression

A test compound is administered to a culture of human cells transfected with a serine palmitoyltransferase expression construct and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells which have not been transfected is incubated for the same time without the test compound to provide a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem.* 18, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ³²P-labeled serine palmitoyltransferase-specific probe at 65°C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO: 1. A test compound which decreases the serine palmitoyltransferase-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of serine palmitoyltransferase gene expression.

EXAMPLE 5

Identification of a test compound which decreases serine palmitoyltransferase activity



A test compound is administered to a culture of human cells transfected with a serine palmitoyltransferase-like enzyme expression construct and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells which have not been transfected is incubated for the same time without the test compound to provide a negative control. Serine palmitoyltransferase activity is measured as described in Weiss & Stoffel, *Eur. J. Biochem.* 249, 239-47, 1997.

A test compound which decreases the serine palmitoyltransferase activity of the serine palmitoyltransferase-like enzyme relative to the serine palmitoyltransferase activity in the absence of the test compound is identified as an inhibitor of serine palmitoyltransferase-like enzyme activity.

EXAMPLE 6

Proliferation inhibition assay: Antisense oligonucleotides suppress the growth of cancer cell lines

The cell line used for testing is the human colon cancer cell line HCT116. Cells are cultured in RPMI-1640 with 10-15% fetal calf serum at a concentration of 10,000 cells per milliliter in a volume of 0.5 ml and kept at 37°C in a 95% air/5%CO₂ atmosphere.

Phosphorothioate oligoribonucleotides are synthesized on an Applied Biosystems Model 380B DNA synthesizer using phosphoroamidite chemistry. A sequence of 24 bases complementary to the nucleotides at position 1 to 24 of SEQ ID NO: 1 is used

as the test oligonucleotide. As a control, another (random) sequence is used: 5'-TCA
ACT GAC TAG ATG TAC ATG GAC-3'. Following assembly and deprotection,
oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate
buffered saline at the desired concentration. Purity of the oligonucleotides is tested
5 by capillary gel electrophoresis and ion exchange HPLC. The purified oligonucleo-
tides are added to the culture medium at a concentration of 10 μ M once per day for
seven days.

The addition of the test oligonucleotide for seven days results in significantly reduced
10 expression of human serine palmitoyltransferase as determined by Western blotting.
This effect is not observed with the control oligonucleotide. After 3 to 7 days, the
number of cells in the cultures is counted using an automatic cell counter. The
number of cells in cultures treated with the test oligonucleotide (expressed as 100%)
is compared with the number of cells in cultures treated with the control
15 oligonucleotide. The number of cells in cultures treated with the test oligonucleotide
is not more than 30% of control, indicating that the inhibition of human serine
palmitoyltransferase has an anti-proliferative effect on cancer cells.

EXAMPLE 7

20

Tissue-specific expression of serine palmitoyltransferase

The qualitative expression pattern of serine palmitoyltransferase in various tissues is
determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). To
25 demonstrate that serine palmitoyltransferase is involved in the disease process of
diabetes, the following whole body panel is screened to show predominant or
relatively high expression: subcutaneous and mesenteric adipose tissue, adrenal
gland, bone marrow, brain, colon, fetal brain, heart, hypothalamus, kidney, liver,
lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle,
30 small intestine, spleen, stomach, testis, thymus, thyroid, trachea, and uterus. Human
islet cells and an islet cell library also are tested. As a final step, the expression of

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serine palmitoyltransferase in cells derived from normal individuals with the expression of cells derived from diabetic individuals is compared.

To demonstrate that serine palmitoyltransferase is involved in cancer, expression is
5 determined in the following tissues: adrenal gland, bone marrow, brain, cerebellum, colon, fetal brain, fetal liver, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid, trachea, uterus, and peripheral blood lymphocytes. Expression in the following cancer cell lines also is determined: DU-145 (prostate),
10 NCI-H125 (lung), HT-29 (colon), COLO-205 (colon), A-549 (lung), NCI-H460 (lung), HT-116 (colon), DLD-1 (colon), MDA-MD-231 (breast), LS174T (colon), ZF-75 (breast), MDA-MN-435 (breast), HT-1080, MCF-7 (breast), and U87. Matched pairs of malignant and normal tissue from the same patient also are tested.

15 To demonstrate that serine palmitoyltransferase is involved in the disease process of obesity, expression is determined in the following tissues: subcutaneous adipose tissue, mesenteric adipose tissue, adrenal gland; bone marrow, brain (cerebellum, spinal cord, cerebral cortex, caudate, medulla, substantia nigra, and putamen), colon, fetal brain, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate,
20 salivary gland, skeletal muscle small intestine, spleen, stomach, testes, thymus, thyroid trachea, and uterus. Neuroblastoma cell lines SK-Nr-Be (2), Hr, Sk-N-As, HTB-10, IMR-32, SNSY-5Y, T3, SK-N-D2, D283, DAOY, CHP-2, U87MG, BE(2)C, T986, KANTS, MO59K, CHP234, C6 (rat), SK-N-FI, SK-PU-DW, PFSK-1, BE(2)M17, and MCIXC also are tested for serine palmitoyltransferase expression.
25 As a final step, the expression of serine palmitoyltransferase in cells derived from normal individuals with the expression of cells derived from obese individuals is compared.

Quantitative expression profiling. Quantitative expression profiling is performed by
30 the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi *et al.*, *BioTechnology* 10, 413-17, 1992, and Higuchi *et al.*, *BioTechnology*

11, 1026-30, 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

5 If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 7276-80, 1991). Because the fluorescence emission will increase in direct
10 proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid *et al.*, *Genome Res.* 6, 986-94, 1996, and Gibson *et al.*, *Genome Res.* 6, 995-1001, 1996).

15 The amplification of an endogenous control can be performed to standardize the amount of sample RNA added to a reaction. In this kind of experiment, the control of choice is the 18S ribosomal RNA. Because reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labeled with different dyes are used.

20 All "real time PCR" measurements of fluorescence are made in the ABI Prism 7700.

RNA extraction and cDNA preparation. Total RNA from the tissues listed above are used for expression quantification. RNAs labeled "from autopsy" are extracted from
25 autoptic tissues with the TRIzol reagent (Life Technologies, MD) according to the manufacturer's protocol.

Fifty µg of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix: 0.2 U/µl RNase-free DNase I (Roche Diagnostics, Germany); 0.4 U/µl
30 RNase inhibitor (PE Applied Biosystems, CA); 10 mM Tris-HCl pH 7.9; 10mM MgCl₂; 50 mM NaCl; and 1 mM DTT.

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After incubation, RNA is extracted once with 1 volume of phenol:chloroform:-isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of 3 M NaAcetate, pH5.2, and 2 volumes of ethanol.

5

Fifty µg of each RNA from the autaptic tissues are DNase treated with the DNA-free kit purchased from Ambion (Ambion, TX). After resuspension and spectrophotometric quantification, each sample is reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) according to the manufacturer's protocol. The final concentration of RNA in the reaction mix is 200 ng/µL. Reverse transcription is carried out with 2.5µM of random hexamer primers.

10

TaqMan quantitative analysis. Specific primers and probe are designed according to the recommendations of PE Applied Biosystems and are listed below:

15

forward primer: 5'-(gene specific sequence)-3'

reverse primer: 5'-(gene specific sequence)-3'

probe: 5'-(FAM)-(gene specific sequence)(TAMRA)-3'

where FAM = 6-carboxy-fluorescein

20

and TAMRA = 6-carboxy-tetramethyl-rhodamine.

The expected length of the PCR product is -(gene specific length)bp.

25

Quantification experiments are performed on 10 ng of reverse transcribed RNA from each sample. Each determination is done in triplicate.

Total cDNA content is normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA using the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

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The assay reaction mix is as follows: 1X final TaqMan Universal PCR Master Mix (from 2X stock) (PE Applied Biosystems, CA); 1X PDAR control – 18S RNA (from 20X stock); 300 nM forward primer; 900 nM reverse primer; 200 nM probe; 10 ng cDNA; and water to 25 µl.

5

Each of the following steps are carried out once: pre PCR, 2 minutes at 50°C, and 10 minutes at 95°C. The following steps are carried out 40 times: denaturation, 15 seconds at 95°C, annealing/extension, 1 minute at 60°C.

10 The experiment is performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR are processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

15

EXAMPLE 8

In vivo testing of compounds/target validation

20 **1. Acute Mechanistic Assays**

1.1. Reduction in Mitogenic Plasma Hormone Levels

25 This non-tumor assay measures the ability of a compound to reduce either the endogenous level of a circulating hormone or the level of hormone produced in response to a biologic stimulus. Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.). At a predetermined time after administration of test compound, blood plasma is collected. Plasma is assayed for levels of the hormone of
30 interest. If the normal circulating levels of the hormone are too low and/or variable to provide consistent results, the level of the hormone

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may be elevated by a pre-treatment with a biologic stimulus (i.e., LHRH may be injected i.m. into mice at a dosage of 30 ng/mouse to induce a burst of testosterone synthesis). The timing of plasma collection would be adjusted to coincide with the peak of the induced hormone response. Compound effects are compared to a vehicle-treated control group. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value ≤ 0.05 compared to the vehicle control group.

1.2. *Hollow Fiber Mechanism of Action Assay*

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol, these may include assays for gene expression (bDNA, PCR, or Taqman), or a specific biochemical activity (i.e., cAMP levels. Results are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at $p \leq 0.05$ as compared to the vehicle control group.

2. *Subacute Functional In Vivo Assays*

2.1. *Reduction in Mass of Hormone Dependent Tissues*

This is another non-tumor assay that measures the ability of a compound to reduce the mass of a hormone dependent tissue (i.e., seminal vesicles in males and uteri in females). Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.) according to a predetermined schedule and for a predetermined duration (i.e., 1 week). At termination of the study, animals are weighed, the target

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organ is excised, any fluid is expressed, and the weight of the organ is recorded. Blood plasma may also be collected. Plasma may be assayed for levels of a hormone of interest or for levels of test agent. Organ weights may be directly compared or they may be normalized for the body weight of the animal. Compound effects are compared to a vehicle-treated control group. An F-test is performed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is $p \text{ value} \leq 0.05$ compared to the vehicle control group.

2.2. *Hollow Fiber Proliferation Assay*

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol. Cell proliferation is determined by measuring a marker of cell number (i.e., MTT or LDH). The cell number and change in cell number from the starting inoculum are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at $p \leq 0.05$ as compared to the vehicle control group.

2.3. *Anti-angiogenesis Models*

2.3.1. *Corneal Angiogenesis*

Hydron pellets with or without growth factors or cells are implanted into a micropocket surgically created in the rodent cornea. Compound administration may be systemic or local (compound mixed with growth factors in the hydron pellet). Corneas are harvested at 7 days post implantation immediately following intracardiac infusion of colloidal carbon and are

fixed in 10% formalin. Readout is qualitative scoring and/or image analysis. Qualitative scores are compared by Rank Sum test. Image analysis data is evaluated by measuring the area of neovascularization (in pixels) and group averages are compared by Student's t-test (2 tail). Significance is $p \leq 0.05$ as compared to the growth factor or cells only group.

2.3.2. *Matrigel Angiogenesis*

Matrigel, containing cells or growth factors, is injected subcutaneously. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Matrigel plugs are harvested at predetermined time point(s) and prepared for readout. Readout is an ELISA-based assay for hemoglobin concentration and/or histological examination (i.e. vessel count, special staining for endothelial surface markers: CD31, factor-8). Readouts are analyzed by Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$ as compared to the vehicle control group.

3. **Primary Antitumor Efficacy**

3.1. *Early Therapy Models*

3.1.1. *Subcutaneous Tumor*

Tumor cells or fragments are implanted subcutaneously on Day 0. Vehicle and/or compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting at a time, usually on Day 1, prior to the ability to measure the tumor burden. Body weights and tumor measurements are recorded 2-3 times weekly. Mean net body and tumor weights

are calculated for each data collection day. Anti-tumor efficacy may be initially determined by comparing the size of treated (T) and control (C) tumors on a given day by a Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$. The experiment may also be continued past the end of dosing in which case tumor measurements would continue to be recorded to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \leq 0.05$.

3.1.2. *Intraperitoneal/Intracranial Tumor Models*

Tumor cells are injected intraperitoneally or intracranially on Day 0. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting on Day 1. Observations of morbidity and/or mortality are recorded twice daily. Body weights are measured and recorded twice weekly. Morbidity/mortality data is expressed in terms of the median time of survival and the number of long-term survivors is indicated separately. Survival times are used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment.

3.2. *Established Disease Model*

Tumor cells or fragments are implanted subcutaneously and grown to the desired size for treatment to begin. Once at the predetermined size

range, mice are randomized into treatment groups. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \text{ value} \leq 0.05$ compared to the vehicle control group.

3.3. *Orthotopic Disease Models*

3.3.1. *Mammary Fat Pad Assay*

Tumor cells or fragments, of mammary adenocarcinoma origin, are implanted directly into a surgically exposed and reflected mammary fat pad in rodents. The fat pad is placed back in its original position and the surgical site is closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine

if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group.

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Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \text{ value} \leq 0.05$ compared to the vehicle control group. In addition, this model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ, or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.2. *Intraprostatic Assay*

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Tumor cells or fragments, of prostatic adenocarcinoma origin, are implanted directly into a surgically exposed dorsal lobe of the prostate in rodents. The prostate is externalized through an abdominal incision so that the tumor can be implanted specifically in the dorsal lobe while verifying that the implant does not enter the seminal vesicles. The successfully inoculated prostate is replaced in the abdomen and the incisions

through the abdomen and skin are closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is performed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the lungs), or measuring the target organ weight (i.e., the regional lymph nodes). The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.3. Intrabronchial Assay

Tumor cells of pulmonary origin may be implanted intrabronchially by making an incision through the skin and exposing the trachea. The trachea is pierced with the beveled end of a 25 gauge needle and the tumor cells are inoculated into the main bronchus using a flat-ended 27 gauge needle with a 90° bend. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are

measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the contralateral lung), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.4. Intracecal Assay

Tumor cells of gastrointestinal origin may be implanted intracecally by making an abdominal incision through the skin and externalizing the intestine. Tumor cells are inoculated into the cecal wall without penetrating the lumen of the intestine using a 27 or 30 gauge needle. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal

5 followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the liver), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

4. Secondary (Metastatic) Antitumor Efficacy

15 4.1. *Spontaneous Metastasis*

Tumor cells are inoculated s.c. and the tumors allowed to grow to a predetermined range for spontaneous metastasis studies to the lung or liver. These primary tumors are then excised. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule which may include the period leading up to the excision of the primary tumor to evaluate therapies directed at inhibiting the early stages of tumor metastasis. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are

- 75 -

compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment for both of these endpoints.

5 4.2. *Forced Metastasis*

Tumor cells are injected into the tail vein, portal vein, or the left ventricle of the heart in experimental (forced) lung, liver, and bone metastasis studies, respectively. Compounds are administered p.o.,
10 i.p., i.v., i.m., or s.c. according to a predetermined schedule. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the
15 other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are compared by Student's t-test
20 after conducting an F-test, with significance at $p \leq 0.05$ compared to the vehicle control group in the experiment for both endpoints.

EXAMPLE 9

25 *Diabetes: In vivo testing of compounds/target validation*

1. Glucose Production

Over-production of glucose by the liver, due to an enhanced rate of gluconeogenesis, is the major cause of fasting hyperglycemia in diabetes. Overnight
30 fasted normal rats or mice have elevated rates of gluconeogenesis as do

streptozotocin-induced diabetic rats or mice fed ad libitum. Rats are made diabetic with a single intravenous injection of 40 mg/kg of streptozotocin while C57BL/KsJ mice are given 40-60 mg/kg i.p. for 5 consecutive days. Blood glucose is measured from tail-tip blood and then compounds are administered via different routes (p.o., i.p., i.v., s.c.). Blood is collected at various times thereafter and glucose measured. Alternatively, compounds are administered for several days, then the animals are fasted overnight, blood is collected and plasma glucose measured. Compounds that inhibit glucose production will decrease plasma glucose levels compared to the vehicle-treated control group.

2. Insulin Sensitivity

Both ob/ob and db/db mice as well as diabetic Zucker rats are hyperglycemic, hyperinsulinemic and insulin resistant. The animals are pre-bled, their glucose levels measured, and then they are grouped so that the mean glucose level is the same for each group. Compounds are administered daily either q.d. or b.i.d. by different routes (p.o., i.p., s.c.) for 7-28 days. Blood is collected at various times and plasma glucose and insulin levels determined. Compounds that improve insulin sensitivity in these models will decrease both plasma glucose and insulin levels when compared to the vehicle-treated control group.

3. Insulin Secretion

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, compounds are administered by different routes (p.o., i.p., s.c. or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4 g/kg) is given, blood is collected one minute

later. Plasma insulin levels are determined. Compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1 g/kg), bled again after 15, 30, 60 and 90 minutes and plasma glucose levels determined. Compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, test compounds which regulate pristanoyl-CoA oxidase-like enzyme are administered by different routes (p.o., i.p., s.c., or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Test compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1 g/kg), bled again after 15, 30, 60, and 90 minutes and plasma glucose levels determined. Test compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

4. Glucose Production

Over-production of glucose by the liver, due to an enhanced rate of gluconeogenesis, is the major cause of fasting hyperglycemia in diabetes. Overnight fasted normal rats or mice have elevated rates of gluconeogenesis as do streptozotocin-induced diabetic rats or mice fed ad libitum. Rats are made

diabetic with a single intravenous injection of 40 mg/kg of streptozotocin while C57BL/KsJ mice are given 40-60 mg/kg i.p. for 5 consecutive days. Blood glucose is measured from tail-tip blood and then compounds are administered via different routes (p.o., i.p., i.v., s.c.). Blood is collected at various times thereafter and glucose measured. Alternatively, compounds are administered for several days, then the animals are fasted overnight, blood is collected and plasma glucose measured. Compounds that inhibit glucose production will decrease plasma glucose levels compared to the vehicle-treated control group.

5. Insulin Sensitivity

Both ob/ob and db/db mice as well as diabetic Zucker rats are hyperglycemic, hyperinsulinemic and insulin resistant. The animals are pre-bled, their glucose levels measured, and then they are grouped so that the mean glucose level is the same for each group. Compounds are administered daily either q.d. or b.i.d. by different routes (p.o., i.p., s.c.) for 7-28 days. Blood is collected at various times and plasma glucose and insulin levels determined. Compounds that improve insulin sensitivity in these models will decrease both plasma glucose and insulin levels when compared to the vehicle-treated control group.

6. Insulin Secretion

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, compounds are administered by different routes (p.o., i.p., s.c. or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4 g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Compounds that enhance insulin

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secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1 g/kg), bled again after 15, 30, 60 and 90 minutes and plasma glucose levels determined. Compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

CLAIMS

1. An isolated polynucleotide encoding a serine palmitoyltransferase polypeptide and being selected from the group consisting of:
- 5
- a) a polynucleotide encoding a serine palmitoyltransferase polypeptide comprising an amino acid sequence selected from the group consisting of:
- 10 amino acid sequences which are at least about 74% identical to the amino acid sequence shown in SEQ ID NO: 2; and the amino acid sequence shown in SEQ ID NO: 2.
- b) a polynucleotide comprising the sequence of SEQ ID NO: 1;
- 15
- c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);
- d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and
- 20
- e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).
- 25
2. An expression vector containing any polynucleotide of claim 1.
3. A host cell containing the expression vector of claim 2.
- 30
4. A substantially purified serine palmitoyltransferase polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a serine palmitoyltransferase polypeptide, wherein the method comprises the following steps:
 - 5 a) culturing the host cell of claim 3 under conditions suitable for the expression of the serine palmitoyltransferase polypeptide; and
 - b) recovering the serine palmitoyltransferase polypeptide from the host cell culture.
- 10 6. A method for detection of a polynucleotide encoding a serine palmitoyltransferase polypeptide in a biological sample comprising the following steps:
 - 15 a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and
 - b) detecting said hybridization complex.
7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.
- 20 8. A method for the detection of a polynucleotide of claim 1 or a serine palmitoyltransferase polypeptide of claim 4 comprising the steps of:
 - 25 contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the serine palmitoyltransferase polypeptide.
9. A diagnostic kit for conducting the method of any one of claims 6 to 8.
- 30 10. A method of screening for agents which decrease the activity of a serine palmitoyltransferase, comprising the steps of:

contacting a test compound with any serine palmitoyltransferase polypeptide encoded by any polynucleotide of claim 1;

5 detecting binding of the test compound to the serine palmitoyltransferase polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a serine palmitoyltransferase.

10 11. A method of screening for agents which regulate the activity of a serine palmitoyltransferase, comprising the steps of:

contacting a test compound with a serine palmitoyltransferase polypeptide encoded by any polynucleotide of claim 1; and

15 detecting a serine palmitoyltransferase activity of the polypeptide, wherein a test compound which increases the serine palmitoyltransferase activity is identified as a potential therapeutic agent for increasing the activity of the serine palmitoyltransferase, and wherein a test compound which decreases the serine palmitoyltransferase activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the serine palmitoyltransferase.

20 12. A method of screening for agents which decrease the activity of a serine palmitoyltransferase, comprising the steps of:

25 contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of serine palmitoyltransferase.

30

13. A method of reducing the activity of serine palmitoyltransferase, comprising the steps of:
- 5 contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any serine palmitoyltransferase polypeptide of claim 4, whereby the activity of serine palmitoyltransferase is reduced.
14. A reagent that modulates the activity of a serine palmitoyltransferase polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.
- 10
15. A pharmaceutical composition, comprising:
- 15 the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.
16. Use of the expression vector of claim 2 or the reagent of claim 14 in the preparation of a medicament for modulating the activity of a serine palmitoyltransferase in a disease.
- 20
17. Use of claim 16 wherein the disease is HIV infection, cancer, obesity, or diabetes.
18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.
- 25
19. The cDNA of claim 18 which comprises SEQ ID NO: 1.
20. The cDNA of claim 18 which consists of SEQ ID NO: 1.
- 30

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.
22. The expression vector of claim 21 wherein the polynucleotide consists of
5 SEQ ID NO: 1.
23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.
- 10 24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO: 1.
25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2.
15
26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO: 2.
27. A fusion protein comprising a polypeptide having the amino acid sequence
20 shown in SEQ ID NO: 2.
28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising the steps of:
- 25 culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and
isolating the polypeptide.
- 30 29. The method of claim 28 wherein the expression vector comprises SEQ ID NO: 1.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising the steps of:
- 5 hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and
- detecting the hybridization complex.
- 10 31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.
32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising:
- 15 a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 1; and instructions for the method of claim 30.
- 20 33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising the steps of:
- contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and
- 25 detecting the reagent-polypeptide complex.
34. The method of claim 33 wherein the reagent is an antibody.
- 30 35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2, comprising:

an antibody which specifically binds to the polypeptide; and

instructions for the method of claim 33.

5

36. A method of screening for agents which can modulate the activity of a human serine palmitoyltransferase, comprising the steps of:

10 contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 74% identical to the amino acid sequence shown in SEQ ID NO: 2 and (2) the amino acid sequence shown in SEQ ID NO: 2; and

15 detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human serine palmitoyltransferase.

37. The method of claim 36 wherein the step of contacting is in a cell.

20 38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

25 40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

30 42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.
44. The method of claim 36 wherein the test compound is bound to a solid support.
45. A method of screening for agents which modulate an activity of a human serine palmitoyltransferase, comprising the steps of:
- contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 74% identical to the amino acid sequence shown in SEQ ID NO: 2 and (2) the amino acid sequence shown in SEQ ID NO: 2; and
- detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human serine palmitoyltransferase, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human serine palmitoyltransferase.
46. The method of claim 45 wherein the step of contacting is in a cell.
47. The method of claim 45 wherein the cell is *in vitro*.
48. The method of claim 45 wherein the step of contacting is in a cell-free system.
49. A method of screening for agents which modulate an activity of a human serine palmitoyltransferase, comprising the steps of:

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contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO: 1; and

5 detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human serine palmitoyltransferase.

50. The method of claim 49 wherein the product is a polypeptide.

10 51. The method of claim 49 wherein the product is RNA.

52. A method of reducing activity of a human serine palmitoyltransferase, comprising the step of:

15 contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 1, whereby the activity of a human serine palmitoyltransferase is reduced.

20 53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 53 wherein the reagent is an antibody.

55. The method of claim 52 wherein the product is RNA.

25

56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.

30 58. The method of claim 52 wherein the cell is *in vitro*.

59. The method of claim 52 wherein the cell is *in vivo*.
60. A pharmaceutical composition, comprising:
- 5 a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2; and
- a pharmaceutically acceptable carrier.
- 10 61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.
62. A pharmaceutical composition, comprising:
- 15 a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO: 1; and
- a pharmaceutically acceptable carrier.
- 20 63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.
64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.
- 25 65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.
66. A pharmaceutical composition, comprising:

an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 2; and

a pharmaceutically acceptable carrier.

5

67. The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO: 1.

10

68. A method of treating a serine palmitoyltransferase dysfunction related disease, wherein the disease is selected from HIV infection, cancer, obesity, or diabetes comprising the step of:

15

administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human serine palmitoyltransferase, whereby symptoms of the serine palmitoyltransferase disfunction related disease are ameliorated.

20

69. The method of claim 68 wherein the reagent is identified by the method of claim 36.

70. The method of claim 68 wherein the reagent is identified by the method of claim 45.

25

71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

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Fig. 1

tatgataagctcattggttgaatcgtttgaggaagcacccttcatgttatggt
 tttcacttacatgggatatggaattggaaccctgtttggctatctcagagact
 ttttaagaaactggggaatagaaaaatgcaacgcagctgtggaagaaaaagaa
 caaaaagattttgtgccactgtatcaagactttgaaaaattttatacaagaaa
 cctttacatgccaatcagagacaactggaaccggcccatctgcagtgtcccccag
 ggcctctgtttgatgtgatggagaggggtatcggacgactataactggacgttt
 aggtttactggaagagtcatacaagatgtcatcaacatgggctcctataactt
 ccttggtccttgcagccaagtatgatgagtcctatgaggacaataaaggatgttt
 tagagggtgtatggcacaggcgtggccagcaccaggcatgaaatgggcaccttg
 gataagcacaaggagttggaggaccttgtggctaagtctcctgaatgtggaagc
 agctatgggtccttgggatgggattcgcaactaactcaatgaatatcccagcat
 tagttggaaagggtatgcctcatttttaagtgatgagttaaaccacacatcgctt
 gtgcttgggggcccgcactctcaggtgcaaccataagaatcttcaaacacaacag
 t

Fig. 2

YDKLIVESFEEAPLHVMVFTYMGYGIGTTFGYLRDFLRNWGIEKCNAVERKE
 QKDFVPLYQDFENFYTRNLYMRIRDNNWRPICSAPGPLFDVMERVSDDYNNWTF
 RFTGRVIKDVINMGSYNFLGLAAKYDESMRTIKDVLEVYGTGVASTRHEMGTL
 DKHKELEDLVAKFLNVEAAMVFGMGFATNSMNI PALVGKGCLILSDELNHTSL
 VLGARLSGATIRIFKHNS

Fig. 3

MRPEPGGCCCRRTVRANGCVANGEVRNGYVRSSAAAAAAGQIHHVTQNGG
 LYKRPFNEAFEETPMLVAVLTYVGYGVLTTFGYLRDFLRWRIEKCHATERE
 EQKDFVSLYQDFENFYTRNLYMRIRDNNWRPICSVPGARVDIMERQSHDYNWS
 FKYTGNIIKGVINMGSYNYLGFARNTGSCQEAATAKVLEEYAGVCSTRQEIGN
 LDKHEELEELVARFLGVEAAMAYGMGFATNSMNI PALVGKGCLILSDELNHAS
 LVLGARLSGATIRIFKHNNMQSLEKLLKDAIVYGQPRTRRPWKKILILVEGIY
 SMEGSIVRLPEVIALKKKYKAYLYLDEAHSIGALGPTGRGVVEYFGLDPEDVD
 VMMGTFTKSFSGASGGYIGGKKELIDYLRTHSHSAVYATSLSPVVEQIITSMK
 CIMGQDGTSLGKECVQQLAENTRYFRRLKEMGFI IYGNEDSPVVPLMLYMPA
 KIGAFGREMLKRNIGVVVVGFPATPIIESRARFCLSAHTKEILDALKEIDE
 VGDLLQLKYSRHLVPLLDLPFDETTYEETED

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Fig. 4

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1  manpgggavc  ngklhnhkkq  sngsqsrnct  kngivkeaqg
   ngkphfydkl  ivesfeeapl
61  hvmvftymgy  gigtlfgylr  dflrnwgiek  cnaaverkeq
   kvrmrtsldl  cqclllskvf
121 sevvmqvqil  esmrcsgtiq  gkfhssppak  phypwaygpv
   ftniswatti  chipn

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Fig. 5

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GTTGCTANTCGTCGCCTACTACTACTACAATGCTGCGAGAAGACGACAGAAGG
GCAGAGACTTTTTTAAGAACTGGGGAATAGAAAAATGCAACGCAGCTGTGGAA
CGAAAAGAACAAAAAGTACGTATGCGCACCTCCCTGGATCTTTGTCAATGCCT
ACTCCTCTCTAAAGTGTTCTCAGAAGTGGTGATGCAGGTGCAGATTCTAGAAA
GCATGAGGTGCTCAGGAACATTTCAGGGCAAATTTTCATTCATCTCCACCTGCT
AAACCCCATTTACCCATGGGCTTATGGACCTGTTTTTACAAACATCTCATGGGC
AACTACTATTTGCCACATACCAAACCTAAGGCTTTTCCCATACCTTGTAATATT
TAATTTTTTGTAACAACCCCATGATATAGATATTAAACTATAATTTTGAAGATA
AAAACATCAAAATATAAAAGCAAGTGCTCCTGGGTGGGACTGTACTTTGGTCT
TCTAGTTGTAAGTTGGGTATTATTTATGCTCTATCCTGAAAAGGAAAGTGCAT
TTGAGTGAACTGGGACTTTTTTTATTTTCATCTAAATCTGATTTCTCACCTTTGA
CACTATTAATATTTTGAACCAATTTTTTTGGGCATTGTAAGAATTTTAACAGCA
TCTGAAAAATATAGCATTAAACCACTAAAGCTAAGGACCCTGATGAAGTCACT
GCACCCCTCCACTACCTCTACTGGAACAGGCACTGGTATNCACTGCTGAGAGAC
CCATAGACGGTTTACATCACANGACTCTGTGGAGACAATCCCCAGTACCCTT
GGACCCGGTAGACTCGTGGGTGGCGAGANCCANAAANAAACACATCCTGCAGT
CACTCCAGGAAGCCCATCCTAGGAAAAGGGGAGAGTATACATCAAGGGAACAC
CCCCANGGACAAAANAATTTGACAACAGTCTTTANNCCCTAAC

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Fig. 6

AACTGCACAAAGAATGGAATAGTGAAGGAAGCCCAGCAAAATGGGAAGCCACA
TTTTTATGATAAGCTCATTGTTGAATCGTTTGAGGAAGCACCCCTTCATGTTA
TGGTTTTCACTTACATGGGATATGGAATTGGAACCTGTTTGGCTATCTCAGA
GACTTTTTTAAGAACTGGGGAATAGAAAAATGCAACGCAGCTGTGGAACGAAA
AGAACA AAAAGATTTTGTGCCACTGTATCAAGACTTTGAAAATTTTATACAA
GAAACCTTTACATGCCAATCAGAGACA AACTGGAACCGGCCCATCTGCAGTGCC
CCAGGGCTCTGTTTGATGTGATGGAGAGGGTATCAGACGACTATAACTGGACG
TTTAGGTTTACTGGAAGAGTCATCAAAGATGT CATCAACATGGGCTCCTATAA
CTTCCTTGGTCTTGCAGCCCAGTATGATGAGTCTATGAGGGACCATAAAGGAT
GTTTTAGAGGTGTATGGGCACANGCGTTGCCAGCACCGGCATGAAATGGGTG
AATTCACCTCATCCTGGCAGAATGCCTCTGTGTTGGAAGGGTCTTGCNAGCTG
ACTCCGTGCCACAGGGGCCACCTGGGTCCTTGGA AAATTGGCAGGCACTCTTN
CCCGTTTGAA

Fig. 7

AGACTGAAAAC TAAGCCTGCAGAGACCTCTGAAGGAAAACCTGTCCCGGGCT
CTGTCACTTCACACCCATGGCTAACCTGGAGGTGGTGCTGTTTGCAACGGGA
AACTTCACAATCACAAGAAACAGAGCAATGGCTCACAAAGCAGAAACTGCACA
AAGAATGGAATAGTGAAGGAAGCCCAGCAAAATGGGAAGCCACATTTTTATGA
TAAGCTCATTTGTTGAATCGTTTGAGGAAGCACCCCTTCATGTTATGGTTTTCA
CTTACATGGGATATGGAATTGGAACCTGTTTGGCTATCTCAGAGACTTTTTA
AGAAACTGGGGAATAGAAAAATGCAACGCAGCTGTGGAACGAAAAGAACAAAA
AGTACGTATGCGCACCTCCCTGGATCTTTGTCAATGCCTACTCCTCTCTAAAG
TGTTCTCAGAAGTGGTGATGCAGGTGCAGATTCTAGAAAGCATGAGGTGCTCA
GGAAC TATTCAGGGCAAATTTTCATTCATCTCCACCTGCTAAACCCCATACCC
ATGGGCTTATGGACCTGTTTTTACAAACATCTCATGGGCAACTACTATTTGCC
ACATACCAAAC TAAGGCTTTTCCCATACTTGTAATATTTAATTTTGTAAACA
ACCCCATGATATAGATATTAAACTATAATTTTGAAGATAAAAACATCAANATA
TAAAAGCAAGTGCTCCCGGGTGGGACTGTACTTTGGGCCTCTAATTGNAATTG
GGTATTATTTATGCNCCATCCCTGAAAAANGGAAAANTGCAATTTNAG

Fig. 8

BLASTP - alignment of genewise_protein_223 against swiss|015270|LCB2_HUMAN

SERINE PALMITOYLTRANSFERASE 2 (EC 2.3.1.50) (LONG CHAIN BASE BIOSYNTHESIS PROTEIN 2) (LCB 2)
 (SERINE-PALMITOYL-COA TRANSFERASE 2) (SPT 2)
 (KIAA0526). //:treml|AB011098|AB011098 1 gene: "KIAA0526"; product: "KIAA0526 protein"; Homo sapiens mRNA for KIAA0526 protein, complete cds.
 //:treml|AF111168|AF111168 5 product: "serine palmitoyl transferase, subunit II"; Homo sapiens serine palmitoyl transferase, subunit II gene, complete cds; and unknown genes. //:treml|Y08686|HSSPTII 1 product: "serine palmitoyl transferase, subunit II"; H.sapiens mRNA for serine palmitoyl transferase, subunit II //:gp|AB011098|3043576 gene: "KIAA0526"; product: "KIAA0526 protein"; Homo sapiens mRNA for KIAA0526 protein, complete cds. //:gp|AF111168|4186182 product: "serine palmitoyl transferase, subunit II"; Homo sapiens serine palmitoyl transferase, subunit II gene, complete cds; and unknown genes. //:gp|Y08686|2564249 product: "serine palmitoyl transferase, subunit II"; H.sapiens mRNA for serine palmitoyl transferase, subunit II.

This hit is scoring at : 2e-96 (expectation value)

Alignment length (overlap) : 230

Identities : 73 %

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Database searched : nrdb

Q: 1 YDKLIVESFEEAPLHVMTYMGYGIGTLEGYLRDFLRNMGIEKCNAAVERKEQKDFVPL
 Y.: . E:FEE.P: V.V.TY:GYG: TLEGYLRDFLR W IEK: A.ER:EQKDFV.L
 H: 55 YKRPNEAFEETPMLVAVLTIVGYGVLTLEGYLRDFLRWRIEKCHATEREEQKDFVSL
 YQDFENFYTRNLVYRIRDNWNRPICSAPGPLEFDMERVSDDYNTWTFRTGRVIKDVINMG
 YQDFENFYTRNLVYRIRDNWNRPICS.PG. .D:MER S.DYNW:F:TC:IK.VINMG
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Fig. 8 (continued)

```

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SYN:LG.A.. . . . . VLE YG.GV.STR.E:G.LDKH:ELE:LVA:FL.VEAAM.
SYNYLGFAARNTGSCQEAATAKVLEFYGAGVCSTRQEIIGNLDKHEELEELVARFLGVEAAMA
FGMGFATNSMNI PALVGKGCCLILSDELNHTSLVLGARLSGATIRIFKHNS      230
:GMGFATNSMNI PALVGKGCCLILSDELNH.SLVLGARLSGATIRIFKHN:
YGMGFATNSMNI PALVGKGCCLILSDELNHASLVLGARLSGATIRIFKHNN      284

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Fig. 9

BLASTP - alignment of gene wise protein_223 against pdb|1BS0|1BS0-A

8-amino-7-oxonanoate synthase(aons, 8-amino-7-ketopelargonate synthase)biological_unit: active as a dimer

This hit is scoring at : 1e-09 (expectation value)

Alignment length (overlap) : 116

Identities : 32 %

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Database searched : nrdb

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    NVEAAMVFGMGFATNSMNI PALVGKGCCLILSDELNHTSLVLGARLSGATIRIFKHN 229
    ...A::F .GFA.N. I.A::K I::D.L:H.SL: .A.LS :.:R F.HN
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SEQUENCE LISTING

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gactataact	ggacgttttag	gtttactgga	agagtcacat	aagatgtcat	caacatgggc	360
tcctataact	tccttggctc	tgcagccaag	tatgatgagt	ctatgaggac	aataaaggat	420
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Leu Arg Asp Phe Leu Arg Asn Trp Gly Ile Glu Lys Cys Asn Ala Ala
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Val Glu Arg Lys Glu Gln Lys Asp Phe Val Pro Leu Tyr Gln Asp Phe
50          55          60

Glu Asn Phe Tyr Thr Arg Asn Leu Tyr Met Arg Ile Arg Asp Asn Trp
65          70          75          80

Asn Arg Pro Ile Cys Ser Ala Pro Gly Pro Leu Phe Asp Val Met Glu
          85          90          95

Arg Val Ser Asp Asp Tyr Asn Trp Thr Phe Arg Phe Thr Gly Arg Val
          100          105          110

Ile Lys Asp Val Ile Asn Met Gly Ser Tyr Asn Phe Leu Gly Leu Ala
          115          120          125

Ala Lys Tyr Asp Glu Ser Met Arg Thr Ile Lys Asp Val Leu Glu Val
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Tyr Gly Thr Gly Val Ala Ser Thr Arg His Glu Met Gly Thr Leu Asp
145          150          155          160

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- 3 -

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Glu Thr Pro Met Leu Val Ala Val Leu Thr Tyr Val Gly Tyr Gly Val
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Leu Thr Leu Phe Gly Tyr Leu Arg Asp Phe Leu Arg Tyr Trp Arg Ile
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- 4 -

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Lys Tyr Thr Gly Asn Ile Ile Lys Gly Val Ile Asn Met Gly Ser Tyr
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Asn Tyr Leu Gly Phe Ala Arg Asn Thr Gly Ser Cys Gln Glu Ala Ala
 180 185 190

Ala Lys Val Leu Glu Glu Tyr Gly Ala Gly Val Cys Ser Thr Arg Gln
 195 200 205

Glu Ile Gly Asn Leu Asp Lys His Glu Glu Leu Glu Glu Leu Val Ala
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Arg Phe Leu Gly Val Glu Ala Ala Met Ala Tyr Gly Met Gly Phe Ala
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Thr Asn Ser Met Asn Ile Pro Ala Leu Val Gly Lys Gly Cys Leu Ile
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 260 265 270

Ser Gly Ala Thr Ile Arg Ile Phe Lys His Asn Asn Met Gln Ser Leu
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Glu Lys Leu Leu Lys Asp Ala Ile Val Tyr Gly Gln Pro Arg Thr Arg
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Arg Pro Trp Lys Lys Ile Leu Ile Leu Val Glu Gly Ile Tyr Ser Met
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Glu Gly Ser Ile Val Arg Leu Pro Glu Val Ile Ala Leu Lys Lys Lys
 325 330 335

Tyr Lys Ala Tyr Leu Tyr Leu Asp Glu Ala His Ser Ile Gly Ala Leu
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His Ser His Ser Ala Val Tyr Ala Thr Ser Leu Ser Pro Pro Val Val
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Lys Leu Ile Val Glu Ser Phe Glu Glu Ala Pro Leu His Val Met Val
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Cys Leu Leu Leu Ser Lys Val Phe Ser Glu Val Val Met Gln Val Gln
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Ile Leu Glu Ser Met Arg Cys Ser Gly Thr Ile Gln Gly Lys Phe His
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790

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 June 2002 (20.06.2002)

PCT

(10) International Publication Number
WO 02/048325 A3

(51) International Patent Classification⁷: **C12N 9/10**,
15/54, 15/63, C12Q 1/68, G01N 33/53, A61K 38/17,
39/395, 31/7088

(21) International Application Number: PCT/EP01/14653

(22) International Filing Date:
13 December 2001 (13.12.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/255,445 15 December 2000 (15.12.2000) US

(71) Applicant (for all designated States except US): **BAYER AKTIENGESELLSCHAFT** [DE/DE]; 51368 Leverkusen (DE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **KOSSIDA, Sophia** [GR/FR]; 28 Rue des 36 Ponts, F-31400 Toulouse (FR).

(74) Common Representative: **BAYER AKTIENGESELLSCHAFT**; 51368 Leverkusen (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,

CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- entirely in electronic form (except for this front page) and available upon request from the International Bureau

(88) Date of publication of the international search report:
14 November 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/048325 A3

(54) Title: REGULATION OF HUMAN SERINE PALMITOYLTRANSFERASE

(57) Abstract: Reagents which regulate human serine palmitoyltransferase and reagents which bind to human serine palmitoyltransferase gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, HIV infection, cancer, obesity, and diabetes.

INTERNATIONAL SEARCH REPORT

Intern: d Application No

PCT/EP 01/14653

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/10 C12N15/54 C12N15/63 C12Q1/68 G01N33/53
 A61K38/17 A61K39/395 A61K31/7088

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

SEQUENCE SEARCH, MEDLINE, BIOSIS, EPO-Internal, WPI Data, PAJ, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEISS ET AL: "Human and murine serine-palmitoyl-CoA transferase. Cloning, expression and characterization of the key enzyme in sphingolipid synthesis" EUROPEAN JOURNAL OF BIOCHEMISTRY, BERLIN, DE, vol. 249, no. 1, October 1997 (1997-10), pages 239-247, XP002108813 ISSN: 0014-2956	1-7, 10-13, 18-65
X	the whole document, especially Fig. 1 -& DATABASE EMBL 'Online! H. sapiens mRNA for serine palmitoyltransferase, 4 October 1997 (1997-10-04) Database accession no. Y08686 XP002211826 70% identity in 692 nt overlap to SEQ ID NO:1 of present application the whole document	1,4
	-/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

2 September 2002

Date of mailing of the international search report

27/09/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5518 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3015

Authorized officer

Herrmann, K

INTERNATIONAL SEARCH REPORT

Intern. Patent Application No.

PCT/EP 01/14653

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>-----</p> <p>WO 02 24884 A (BAYER AG ;KOSSIDA SOPHIA (US)) 28 March 2002 (2002-03-28)</p> <p>SEQ ID NO:6 is 100% identical in 690 nt overlap to SEQ ID NO:1 of present application</p> <p>-----</p>	<p>1-7, 10-13, 18-65</p>

INTERNATIONAL SEARCH REPORT

In international application No.
PCT/EP 01/14653

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 13, 36, 37, 40-46, 52-57, 59, 62, 68-71 are directed to a method of treatment of the human/animal body or to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 8, 9, 14-17, 68-71 and partially claims 1-7, 10-13, 33, 35, 49-60, 62-65

Claims 1-7, 10-13 (partially):

The subject-matter of claim 1(d) is directed to a polynucleotide which deviates from the polynucleotide sequence specified in claim 1(a-c) due to the degeneration of the genetic code. Reference to the degeneracy of the genetic code only makes sense if the peptide for which the polynucleotide is to encode for is defined by its exact amino acid sequence. However, this is not the case in claim 1(d). Thus, a reasonable search could not be performed for the subject-matter of claim 1(d) (Art. 6 PCT). Consequently, this objections also applies to the subject-matter of claims 2-7 and 10-13, which refers to the insufficiently defined polypeptide of claim 1(d) (Art. 6 PCT).

Claim 9:

Claim 9 contains no technical features which would enable a reasonable search (Art. 6 PCT).

Claims 8, 14-17, 68-71:

Compounds as such are not sufficiently defined by their mode of action. Therefore, claims 8, 14-17 and 68-71 have not been searched because "a reagent that modulates the activity/function of or a reagent which specifically interacts with a serine palmitoyltransferase polypeptide or a polynucleotide" is neither disclosed nor supported within the terms of Art. 5 and 6 PCT, respectively (Art. 17(2)(a)(ii) PCT).

Claims 13, 33, 35, 52, 53, 55, 58-60, 62 (partially):

Compounds as such are not sufficiently defined by their mode of action. Therefore, claims 13, 33, 35, 52, 53, 55, 58-60 and 62 have been searched partially because "a reagent that specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2" other than an antibody, an antisense oligonucleotide or a ribozyme is neither disclosed nor supported within the terms of Art. 5 and 6 PCT, respectively (Art. 17(2)(a)(ii) PCT).

Claims 49-51 and 52-59, 62-65 (partially):

Independent claims 49, 52 and 62 cover all products encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO:1. The number of such products is virtually infinite (Art. 5 and 6 PCT). Thus, a meaningful search over the whole of the scope claimed is impossible. Consequently, the search has been limited to the polypeptide according to SEQ ID NO:2 and an RNA strand which corresponds to the sequence of SEQ ID NO:1 over the whole length.(cf. Art. 17(2)(a)(ii) PCT).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/EP 01/14653

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 0224884	A	28-03-2002	AU	1224602 A		02-04-2002
			WO	0224884 A2		28-03-2002